Technical Report No. IRL 1050

CYTOCHEMICAL STUDIES OF PLANETARY MICROORGANISMS EXPLORATIONS IN EXOBIOLOGY

Status Report Covering Period September 1, 1965 to April 1, 1966
For
National Aeronautics and Space Administration
Grant NsG 81-60

Instrumentation Research Laboratory, Department of Genetics
Stanford University School of Medicine
Palo Alto, California

Report to the National Aeronautics and Space Administration
"Cytochemical Studies of Planetary Microorganisms - Explorations in Exobiology"

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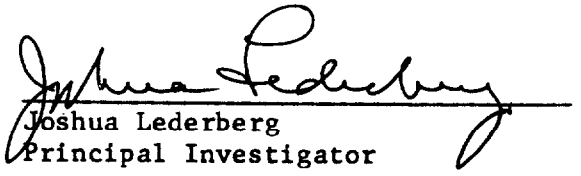

Joshua Lederberg
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Director, Instrumentation Research Laboratory

A. INTRODUCTION

This Status Report covers the activities of the Instrumentation Research Laboratory from September 1, 1965, to April 1, 1966. Major technical efforts are described in separate technical reports and papers. The status report refers to these and summarizes continuing projects.

At the end of this report period we completed the move to the new laboratory facilities provided by NASA Grant NsG-(F)-2. Work under grant NsG 81-60 includes areas of research that are closely related to efforts being carried out in the Department of Genetics under other grants or contracts. This includes Air Force Contract AF 49(638)1599 for "Molecular Biology Applications of Mass Spectroscopy," National Institute of Neurological Diseases and Blindness Grant NB-04270 entitled "Molecular Neurobiology" and a MACY Foundation Grant for "Planning for Advanced Computer Facility - The Stanford Medical School." Work under this latter grant is preparatory to carrying out the Advanced Computer for Medical Research (ACME) proposal submitted to the National Institute of Health, Division of Research Facilities and Resources. In addition, there is collaboration with the work in the Computer Science Department on artificial intelligence carried out under support of the Advanced Research Projects Agency SD 183. The relationship of the work carried out under NASA grant to these other activities has continued to prove of great mutual benefit in all cases.

The general project areas of the Resume are:

I. Multivator
II. Fluorometry
III. Gas Chromatograph and Optical Resolution
IV. Mass Spectrometry
V. Computer Managed Instrumentation
VI. UV Microspectrometry

During the six month period described above, three technical reports were published and five papers submitted to journals for publication. A listing of these reports, arranged in chronological order, is included in this status report. Information covering personnel changes is presented.

The technical efforts relating to this grant (NsG 81-60) extend to the following research areas:

1. Development of specific, mission-oriented experiments for planetary explanation - e.g., the Multivator and the Pasteur Probe.

2. Studies of analytical systems that have special potential in long-range plans for an automated laboratory for Mars, i.e., Mass Spectrometry and Phosphorimetry.

3. Automation of laboratory science under computer control - e.g., LINC computer programming systems; data acquisitions and reduction and closed loop instrument control; mechanized inference from experimental data (artificial intelligence).

The prime responsibility for performance of the research effort under this grant rests with the Instrumentation Research Laboratory which functions as part of the Stanford University School of Medicine Department of Genetics. In addition, other participating groups include Professors Carl Djerassi (Chemistry Department), Lubert Stryer (Biochemistry), Peter Bulkeley (Director, Design Division, Mechanical Engineering), and John McCarthy and Edward Feigenbaum (Computer Sciences), and their associates.

B. PROGRAM RESUME

I. Multivator: Evaluation of a Neon Light Source for Phosphatase

In a previous communication (Technical Report No. IRL-1027) it was noted that, for a number of reasons, unacceptably poor levels of fluorescent detection of 6,6'-dihydroxy-napthofluoran (N.F.) were obtained. In brief these reasons were (1) inadequate output of the tungsten source used in the desired spectral band, (2) excessive scattering of the excitation light by the mechanical housing and the cuvette, (3) imperfect collimation of the source output, occasioned by the requirement that physical dimensions be kept to a minimum, (4) less than optimum spectral response from the multiplier phototube used.

An assembly has been devised which removes or reduces these limitations to the point where the lower limit of detection is close to 10^{-10} molar concentration of N.F. compared to the previously obtained 10^{-8}.

Figures 1 and 2 show the physical arrangements of the assembly. The tungsten filament lamp used as the source in previous experiments has been replaced by a small neon lamp, type N.E.2. operated at a power dissipation of 112 milliwatts, and modulated at 120 cps.

Three graded apertures following the neon lamp and primary interference filter (Optics Technology 595mμ) permitted a divergent cone of light to illuminate the bottom of a 5 cc. cuvette. The cone angle was limited such that total internal reflection of the marginal rays occurred within the cuvette, enabling improved utilization of excitation power. The cuvette was located at the center of a semi-circular polished aluminum reflector and masked at the top and bottom. The fluorescent activity occurring within the central unmasked portion of the cuvette, representing a volume of 2 cc., was detected by the red sensitive multiplier phototube (R.C.A. type 4463) after transmission through a secondary filter (Corning glass type CS2-58).

The output of the multiplier phototube was electrically filtered and amplified by an operational amplifier with the feedback network, arranged in parallel tee configuration. The gain in this mode was 2×10^3 and the bandwidth a few cycles per second. The output of the amplifier was measured with a vacuum tube

voltmeter (H.P. 400-H).

Alternate comparisons were made between an 0.1 concentration of NaOH, used as a reference, and various concentrations of N.F. by substitution of cuvettes.

One set of readings, typical of many obtained, was:

0.1 NaOH	- 0.35 MV	A.C.	R.M.S.
10^{-9} N.F.	- 0.65 "	"	"
10^{-8} N.F.	- 2.1 "	"	"
10^{-7} N.F.	-10.1 "	"	"
10^{-6} N.F.	-92.0 "	"	"

The short term stability of the system was such that identical measurements were made of the above concentrations after an interval of two hours. It had been noted that cuvettes, after routine handling and washing, develop numerous small scratches and surface markings. A test was made to evaluate the possible data spread caused by scattering at these optical inhomogeneities. Six cuvettes were taken at random from a group of twenty-one, filled with an 0.1 NaOH concentration and comparisons made between them. Within the limits of the system sensitivity no differences could be detected between the six cuvettes.

The dimensions of the instrument described are certainly not consistent with the requirements of practical flight hardware, but the majority of system elements may readily be miniaturized. The amplifier module used has a volume of 13 cc., excluding six components which form an external feedback loop. The volume of this unit could be reduced to about 0.3 cc., including external loop, with zero or minimal sacrifice in electrical parameters.

The multiplier phototube supply used, although small by previous industrial standards, is gross compared to anticipated mission hardware. Since the power requirement of the phototube is so low, nominally one one-hundredth of a watt, it should be feasible to reduce this supply unit to a volume of less than 50 cc. The multiplier phototube used, measuring 15 cms. long by 5 cms. diameter, is considered to be too large for use in a practical mission; this particular tube was selected only for its spectral characteristics and a ruggedized type of appropriate configuration, and reduced dimensions should be available on special order.

The power supplies for the neon lamp and the amplifier are considered to be sufficiently nonspecialized to present no dimensional problem; both fall into a generally usable range. Weights and volume consideration can better be evaluated when it is known whether multivator is to be a self contained experiment or auxiliary to other experimentation.

The foregoing indicates that an adequately sensitive phosphatase assay may be made using a miniature neon source in an appropriate physical configuration. Sensitivity obtained was about twice that of a bench model fluorimeter (Turner II) used for comparison and calibration purposes. The sensitivity of the present system could be improved by an order of magnitude of a measurement period of twenty seconds could be tolerated, and if the two filters and detector were more carefully selected.

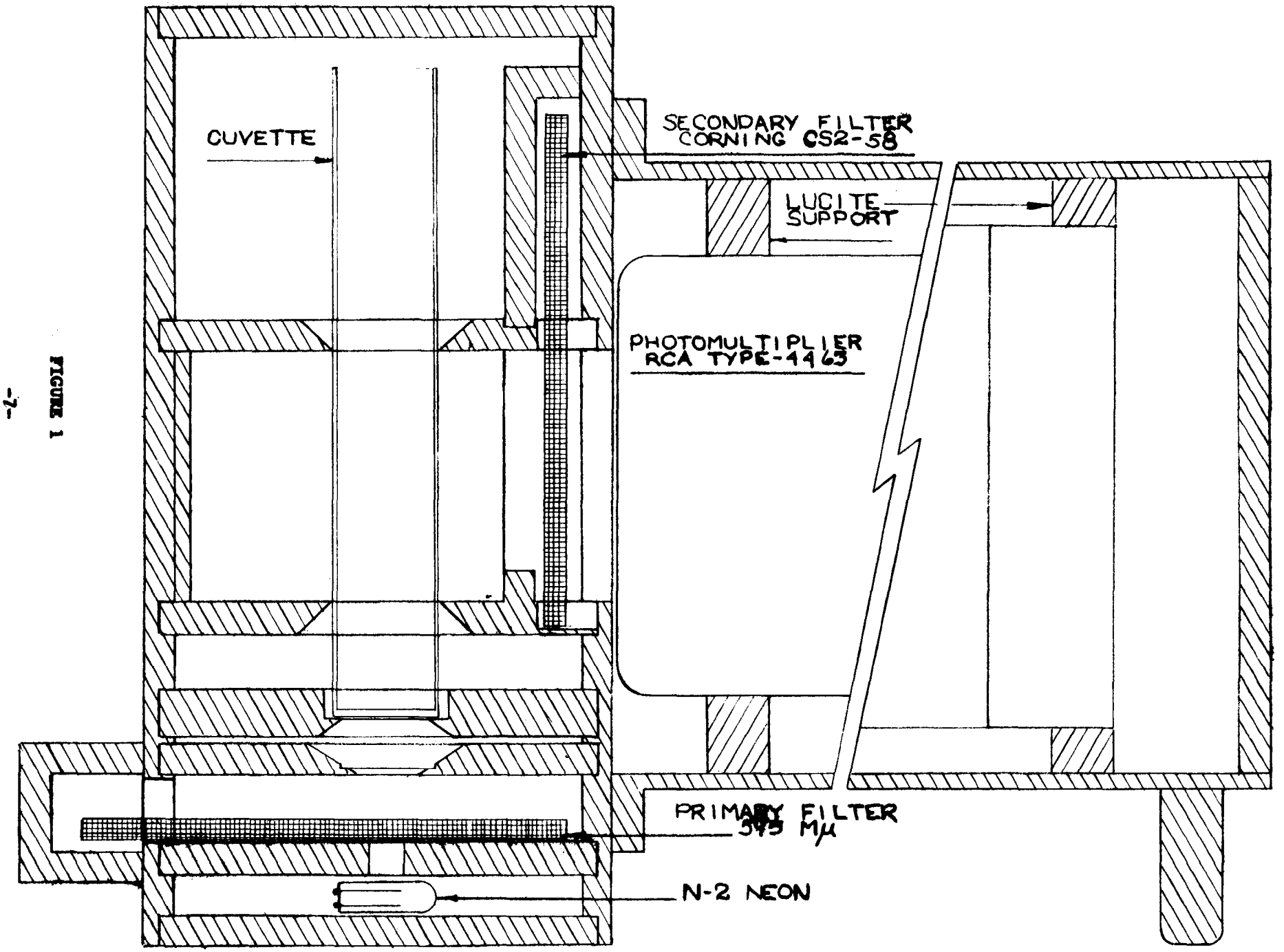


FIGURE 1

J. J. Gilderoy Jr.
2-11-66

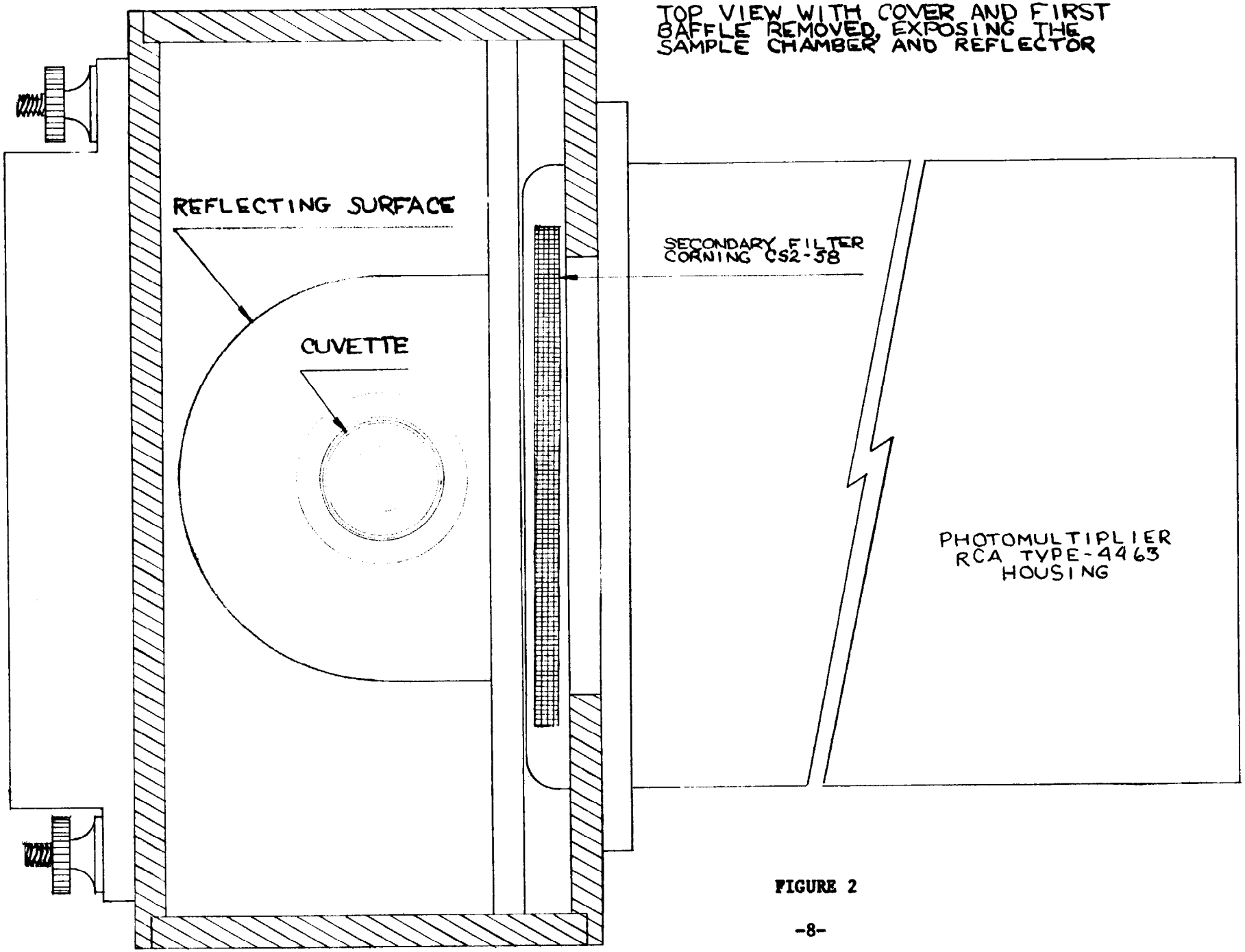


FIGURE 2

J.J. Gilderoy, Jr
2.11.46

II. Fluorometry

a. Reagents for Fluorometric Assays of Hydrolytic Enzymes.

The B-napthylamides of nine different amino acids have been tested as fluorogenic substrates for peptidase activity in soil. The three soils tested (10 mg. soil in 10 ml. solution) all showed activity in 1-2 hours and each showed quite distinct specificity. For instance, Bowers Clay (from NASA Ames) showed greatest activity towards lysyl-B-naphthylamide, whereas desert soil from Panamint Valley exhibited preferential attack on the phenylalanyl derivative. Heating the soil at 130° diminished the activity confirming that it was due to the presence of micro-organisms. These results are described in Table I.

We have now selected and purified four different strains of bacteria from Bowers Clay and intend to investigate the specificity of these pure strains and also to determine the quantitative turnover of substrate per bacterium.

In cooperation with Dr. Lubert Stryer of the Department of Biochemistry, Stanford University, work is continuing on the development of a fluorogenic substrate that could function for a broad array of enzymes.

We have shown that energy can be transferred from a fluorescent group F to a quencher Q over distances of some 20Å. In the model system studied, F and Q are naphthalene derivatives, while the "rigid stick" separating them is a polypeptide helix. This suggests a general type of assay for bond-cleaving enzymes. The fluorogenic substrate is a molecule F-S-B-X-Q, where B is a susceptible bond, and X is a spacer group. Following bond cleavage, F is no longer quenched, since in dilute solutions it is separated from Q by a distance greater than 50 Å. Preliminary studies have shown that quenchers such as the dinitrophenyl group act as inhibitors of certain enzymes. In further work on a general fluorogenic substrate, it will be necessary to (1) choose F and Q which are not enzyme inhibitors and (2) separate F and Q from the susceptible bond B by use of appropriate spacer groups.

b. Nanosecond Flash Fluorometry (Phosphorometry)

As previously reported, a flash fluorometer has been developed in this laboratory in cooperation with Dr. Lubert Stryer of the Department of Biochemistry. In addition to the work reported here some of this research is described in the progress report under grant NGR-05-020-137 covering period January 1, 1966, to

RATES OF HYDROLYSIS OF AMINO ACID β-NAPHTHYLAMIDES*

	Neutral Amino Acids						Hydroxy	Acidic	Basic
	alanine	valine	proline	leucine	Isoleu	Phe	Serine	Glu	Lys
Non-enzymatic hydrolysis	0.0047	0.0028		0.0012	0.0033	0.0033	0.0035	0.001	0.0036
Bowers Clay # 2 (10^5 bacteria per 10mg.)	1.41	0.44	0.52	2.14	0.27	1.47	0.72	0.1	2.25
San Mateo Soil	0.17	0.18	0.23	0.85	0.48	0.61	0.17	0.18	0.47
Panamint Valley Desert Soil	1.2	0.36	0.60	1.3	0.35	1.75	0.34	0.17	0.73

*Rates of hydrolysis in mμ M per hour per 10mg. soil per 10ml. solution.

TABLE I

June 30, 1966, by Professors A. Kornberg and L. Stryer and a paper entitled "A Spin-Labeled Hepten" (L. Stryer and O. Griffiths), Proc. Nat. Acad. Sci. U.S. 54 1785-1791 (1965).

Further work on the system has resulted in shorter and brighter light pulses and it seems apparent that we do not yet know the inherent limit of the system. Presently, photomultiplier transit time spread is the limiting factor.

The light source consists of a high pressure lamp in a coaxial housing with a resistor string. The lamp with the resistors and the distributed capacity of the lamp to housing form a relaxation oscillator for generating the light pulses.

The lamps that have been investigated are xenon, oxygen, hydrogen, and various combinations of the latter two with xenon. With the molecular gasses, the pulse length is unknown as the phototube limits the system at 3 to 4 x 10^{-9} sec. The energy input is a few microjoules per flash. In the case of xenon and combinations containing xenon, the pulse has a half-width on the order of 8 to 10 x 10^{-9} sec. and power input of 1 to 10 millijoules. This very bright flash is excellent for many biochemical experiments.

The lamps used were manufactured by P.E.K. Labs. of Sunnyvale, California. Some of the experimental results were independently verified by Dr. Edwin Garwin of the Stanford Linear Accelerator Center. Reports of both the apparatus and biochemical results to date are to be published shortly.

III. Gas Chromatography and Optical Resolution

Since biogenetic manomolecules have the necessary information to discriminate among the optical isomers of momomeric substrates, this property can be used to ascertain the presence of biological agents in planetary soils. Using the high sensitivity g.l.c. technique previously described, the stereo specific consumption of several racemic amino acid substrates in terrestial soils has been demonstrated. Work is continuing on basic chemical refinements of the techniques. The Pasteur Probe experiments are being studied with specific organisms isolated from the soils previously studied.

The g.l.c. technique has also been used extensively to investigate the stereospecific action of several pure enzymes. In some cases, the results of this study have led to the assignment of absolute configurations to the antipodes of organic compounds such as the alcohols and the amines.

This work is reported in more detail in reports (2,3) and papers (1-5).

IV. Mass Spectrometry.

a. Analysis of Natural Products.

The Atlas CH-4 Mass Spectrometer in Professor Djerassi's laboratory in the Department of Chemistry has yielded the results reported in the following papers:

Djerassi, C.; Sample, S.D.: Mass Spectrometry in Structural and Stereochemical Problems. Mass Spectrometric Fragmentation of Nitrophenylhydrazones. Nature, 308, 1314 (1965).

Djerassi, C.; Shapiro, R. H.; and Vandewalle, M.: Mass Spectrometry in Structural and Stereo-chemical problems. Stereospecificity in Hydrogen Transfer Reaction Characteristic of 6-Keto Steroids. J. Am. Chem. Soc., 87, 4892 (1965).

Duffield, A. M.; Aplin, R. T., Budzikiewicz, H.; Djerassi, C.; Murphy, C.F.; and Wildman, W. C.: Mass Spectrometry in Structural and Stereo-chemical Problems. A Study of the Fragmentation of Some Amaryllidaceae Alkaloids.

Poisson, J.; Plat, M.; Budzikiewica, H.; Durham, L. J.; and Djerassi, C.: Mass Spectrometry in Structural and Stereo-chemical Problems. Partial Structure of Vobtusine.

Djerassi, C.; and Fenselau, C.: Mass Spectrometry in Structural and Stereochemical Problems. The Nature of the Cyclic Transition State in Hydrogen Rearrangements of Aliphatic Amines. J. Am. Chem. Soc., 87, 5747 (1965).

Djerassi, C. and Fenselau, C.: Mass Spectrometry in Structural and Stereochemical Problems. The Nature of the Cyclic Transition State in Hydrogen Rearrangements of Aliphatic Amines. J. Am. Chem. Soc., 87, 5752 (1965).

b. Mass Spectral Microanalysis of Solids

Construction of the ion bombardment solids mass spectrometer has been completed. The desired experiments have been performed and the data has been evaluated.

The instrument comprises a Slodzian-Castaing source unit containing a primary ion gun, a sample holder and inlet system, and secondary ion extraction and focusing optics. There are additionally a flight tube, electron multiplier ion detector, 60° sector bending magnet, and associated vacuum equipment and electronic instrumentation.

Target material is sputtered by the incidence of nominal 10 kev inert gas ions. Approximately $1:10^5$ of the emitted target particles are evolved in ionized form. These secondary ions are extracted from the target region and fired down the flight tube into the field of the bending magnet wherein the approximately monoenergetic ions are fanned out in accordance with their charge to mass ratio. Those ions bent into an 8" radius of curvature trajectory in the magnet pass through an exit slit onto an electron multiplier. The mass spectrum is scanned by sweeping the magnetic field.

Slodzian has demonstrated that, by introducing an appropriately figured magnet and suitable ion-electron image converting optics, the secondary ions may be focused to produce magnified images indicative of the atomic distribution on the surface. One micron resolution has been achieved.

The intention of the present study was to ascertain if sufficient numbers of intact molecular ions characteristic of organic target materials could be evolved to enable the determination of the molecular distribution in structures of biological interest.

Positive primary ions of argon and hydrogen were employed to bombard polyethylene, nylon, polyvinalpyrolidone methylcellulose, graphite, OFHC copper, gold, and aluminum. Both positive and negative secondary ions were examined. The incident primary ion energy was approximately 6 kev and 14 kev during the examination of positive and negative secondaries, respectively.

The molecules evolved from the bombarded organic samples were found generally to constitute rearranged configurations not representative of the original sample structure. It seems probable that the incident primaries produce sufficient local dissipation of energy to devastate the molecular structure. Consequently, the "hottest" of the evolved secondaries come off as rearranged molecular ions and the majority of the material recondenses on the surface, subsequently to be re-evolved, retaining little information regarding the original structure.

As often happens there has been an unexpected finding in this work. At one state in the data analysis it was decided to plot only those mass peaks in any given run that corresponded to singly ionized integral numbers of carbon atoms clumped together. It was thought that this information might provide a useful clue as to the nature of the processes taking place at the surface. When such plots were made for negative secondaries, a distinct pattern revealed itself for all observed combinations of primary ions and organic targets. Namely, there was a preference for the negative secondaries to contain an even number of carbon atoms. This effect has been seen to exist out to 12 carbon atoms, whereupon the effect faded out into the "noise." The effect has been found to persist with the addition of one or two protons to the complex. When graphite was examined it was found that the curve corresponding to one proton affixed to the complex was a replica of the unprotonated curve but displaced downward, on a semilog plot. The addition of two protons, however, so enhanced the effect that it could be observed out to 14 carbons and over 6 decades of intensity whereupon instrumental sensitivity limited further observation. A typical odd-even enhancement in intensity was 10-fold.

A complimentary but less pronounced enhancement of odd carbon clumps over evens has been found for positive secondaries.

Arguments based on observed odd-even carbon effects have even recently been offered in the literature in support of the biogenic origin of oil; the rationale being that such preferential effects mediate in favor of an ordered production mechanism. That odd-even carbon effects can be generated by a-biogenic mechanisms would appear to weaken this particular argument.

Odd-even carbon effects have been reported in the past. They appear, however, not to be widely known. Since the only explanation we can find for these effects calls for the carbon complexes to be in the form of linear chains, we are forced to conclude that most of the evolved ionized carbon complexes are in such form.

Other methods of producing ionized fragments from biological targets that would be useful for microidentification are under study.

c. Mass Spectrometer Data Presentation.

The PMOD-14" plot implements a suggestion that if a spectrum of some organic material is displayed on a spiral base, instead of a conventional orthogonal coordinate system, the influence of CH_2 would be apparent. Two compounds that differ by one CH_2 (14 amu), homologs, would have similar patterns that would be visually apparent. Also the residue, or non-dependent portion would aid visually in the identification of the class of the compound.

The plot program is a computer output package for displaying the display of the spectra. Present input is via punched cards, but it can be adapted to any computer process system with a plotter. This program accepts a list of paired numbers, mass/charge ratio and amplitude, and prepares a conventional orthogonal plot and a spiral based plot of the same data. The plot format is suitable for publication. In the spiral plot, each mass is represented as a filled in triangle whose area is proportional to the amplitude of that mass. The angular position of a given mass is (M/q MOD 14) x 2 x π. The radial position is (M/q + 1) x constant. Thus any two mass peaks differing by 14 will lie on the same radial position but on adjacent laps of the spiral. Any two masses different by x x 14 mass units will lie on the same radial. The mass spectra previously investigated* are being plotted in this form. Examples of Glycine, Valine, Leucine and Isoleucine are shown in Figures 3A through 6B.

*N. Martin, "An Investigation of the Mass Spectra of Twenty-two Free Amino Acids." IRL No. 1035, September 21, 1965.

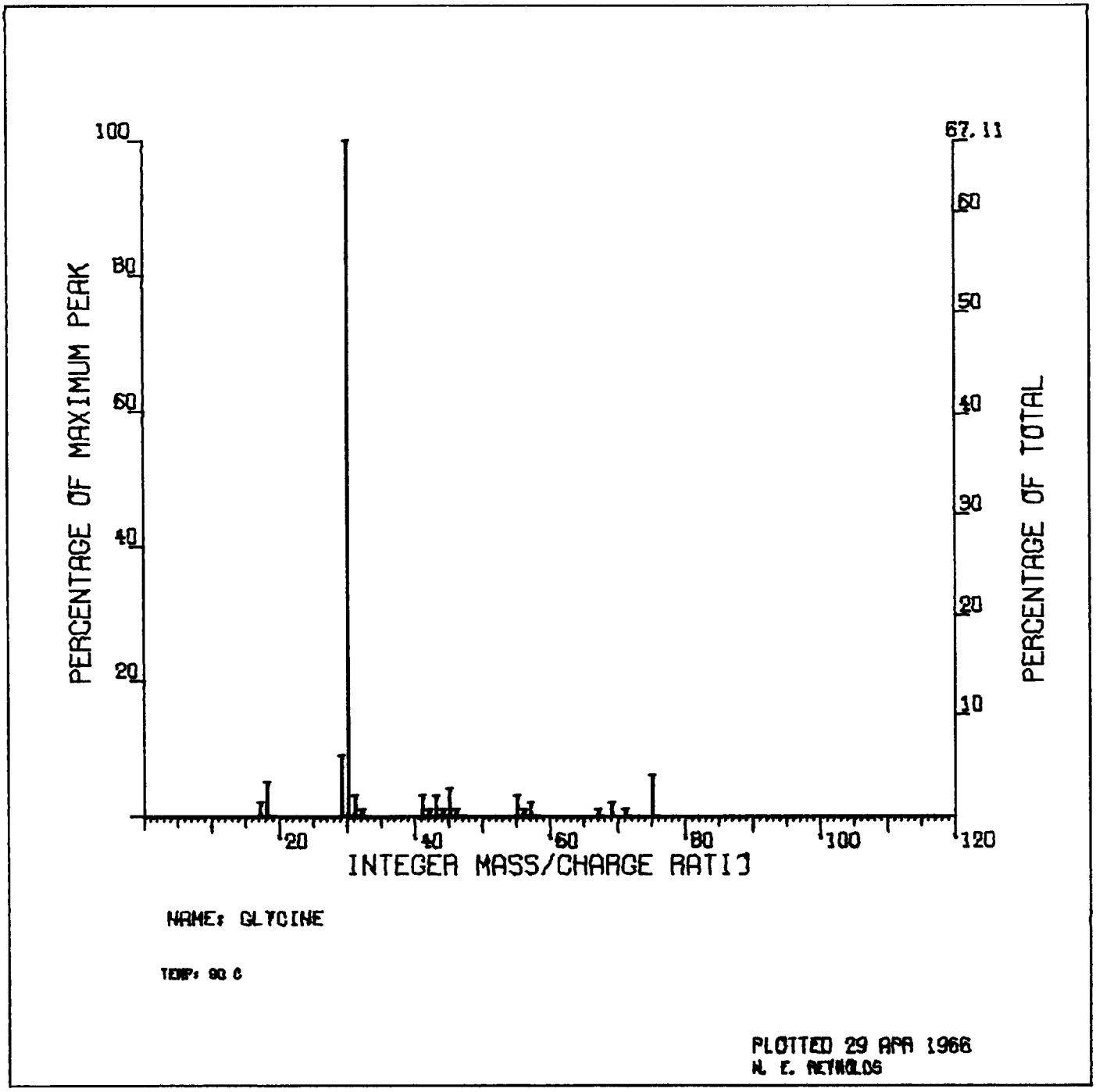
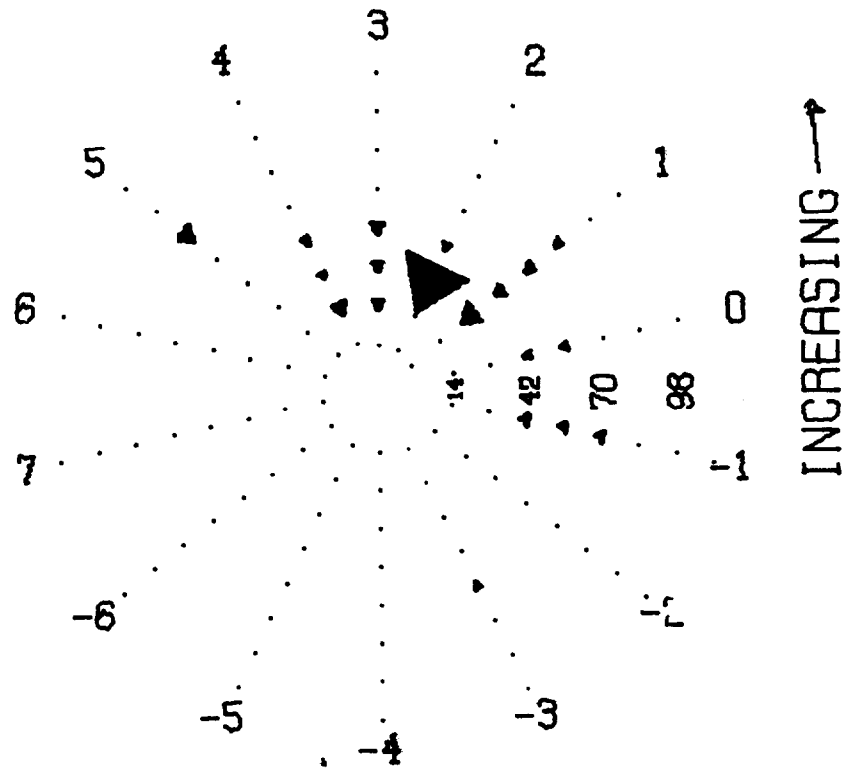

100
80
60
40
20
PERCENTAGE OF MAXIMUM PEAK
67.11
60
50
40
30
20
10
PERCENTAGE OF TOTAL
20
40
60
80
100
120
INTEGER MASS/CHARGE RATIO
NAME: GLYCINE
TEMP: 90 C
PLOTTED 29 APR 1966
N. E. REYNOLDS

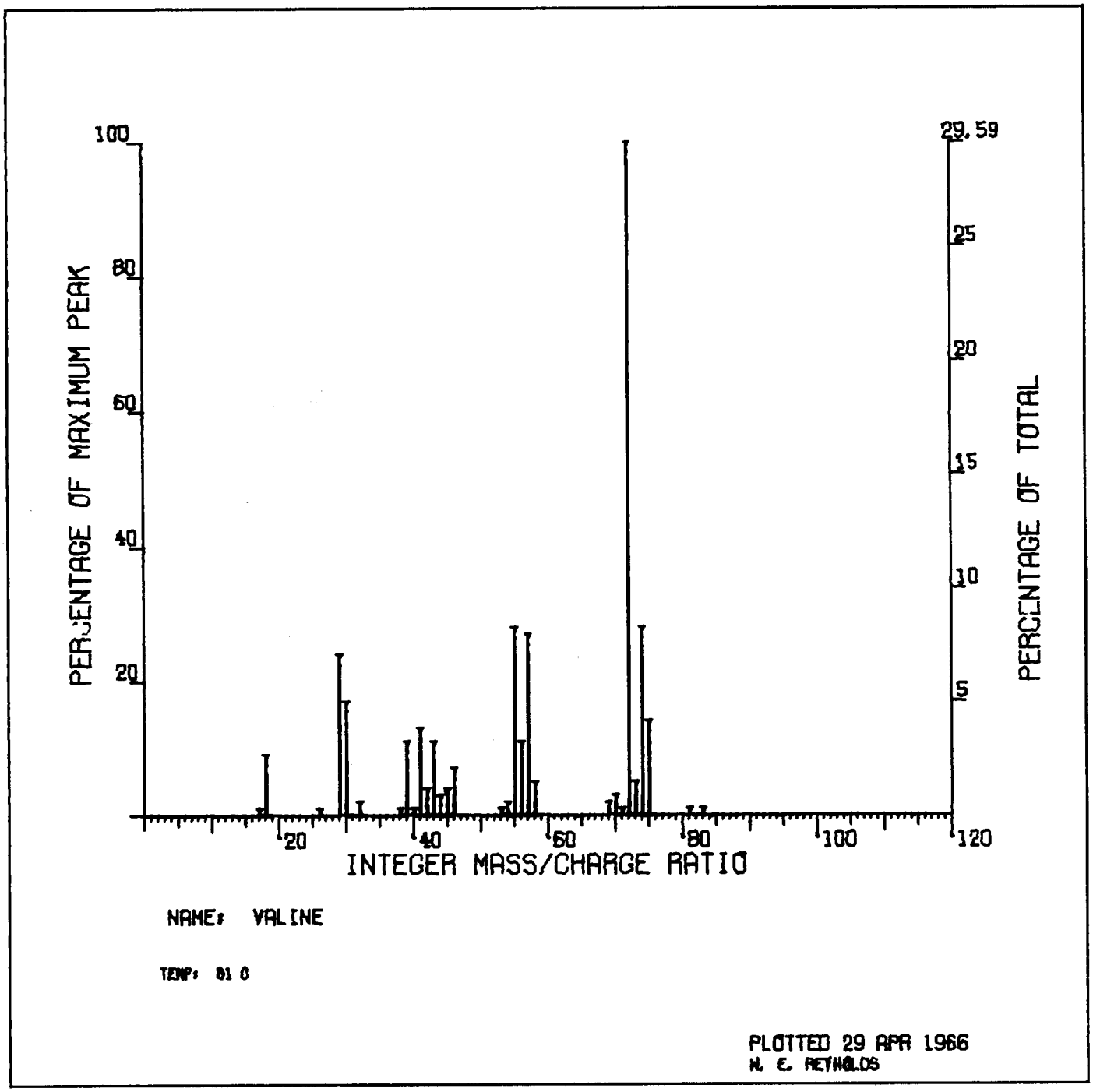
FIGURE 3A

NAME: GLYCINE



TEMP: 90 C

FIGURE 3B


100
80
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20
PERCENTAGE OF MAXIMUM PEAK
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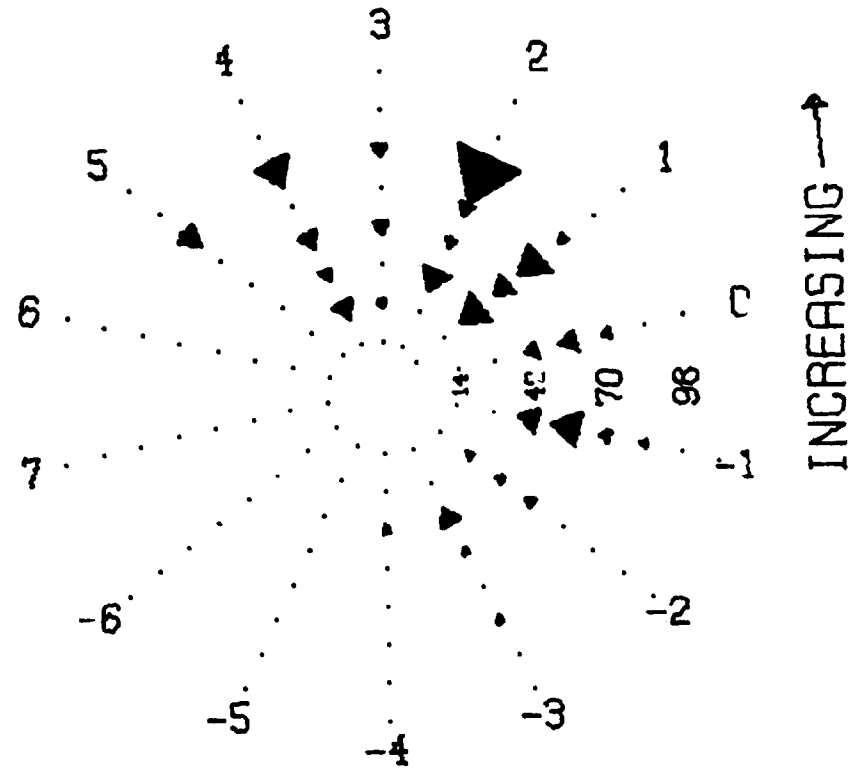
25
20
15
10
5
PERCENTAGE OF TOTAL
20
40
60
80
100
120
INTEGER MASS/CHARGE RATIO
NAME: VALINE
TEMP: 81 C
PLOTTED 29 APR 1966
N. E. REYNOLDS

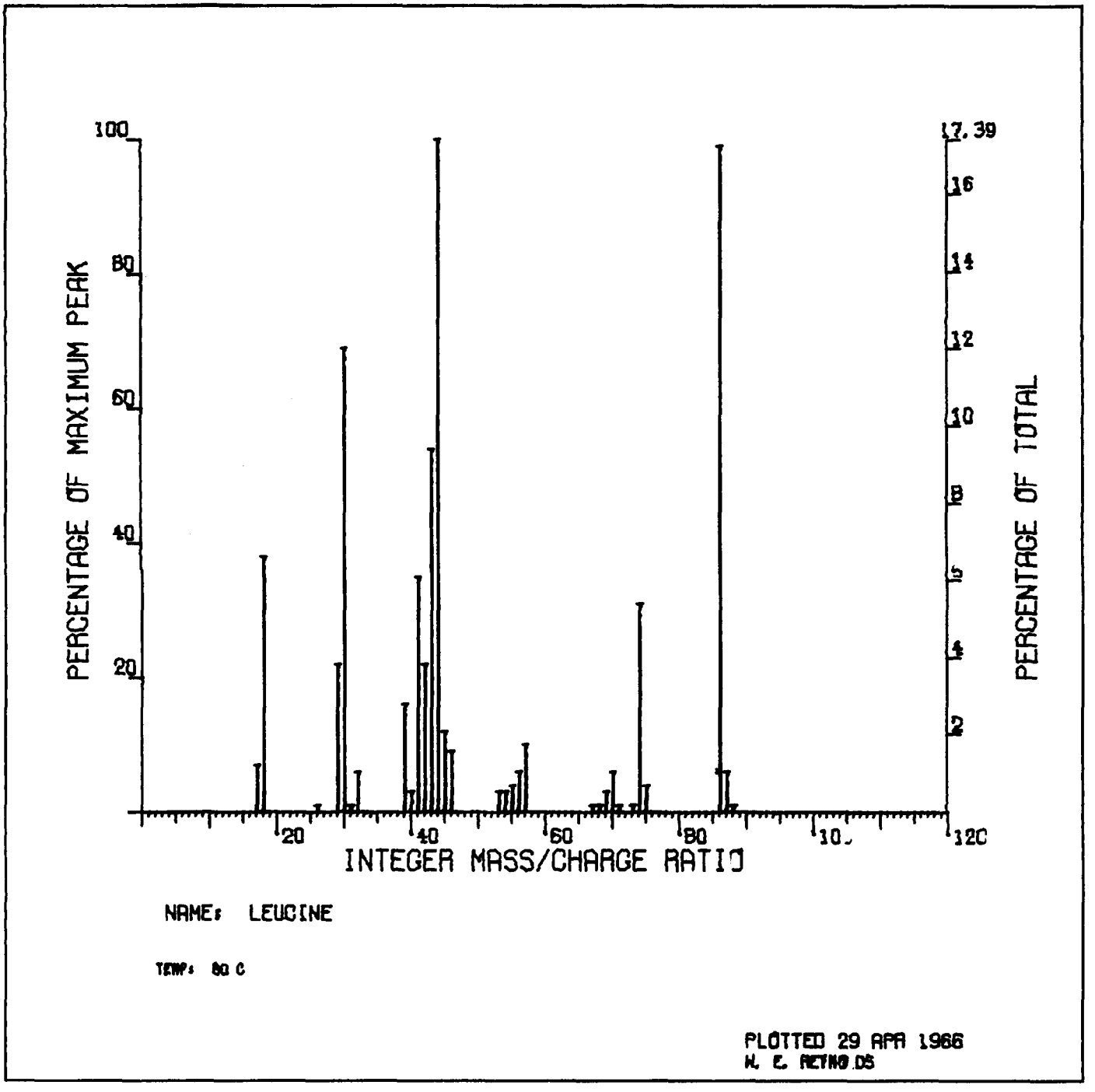
FIGURE 4A

NAME: VALINE



TEMP: 81 C

FIGURE 4B


100
80
60
40
20
PERCENTAGE OF MAXIMUM PEAK
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16
14
12
10
8
6
4
2
PERCENTAGE OF TOTAL
20
40
60
80
120
INTEGER MASS/CHARGE RATIO
NAME: LEUCINE
TEMP: 80 C
PLOTTED 29 APR 1966
M. E. REYNOLDS

FIGURE 5A

NAME: LEUCINE

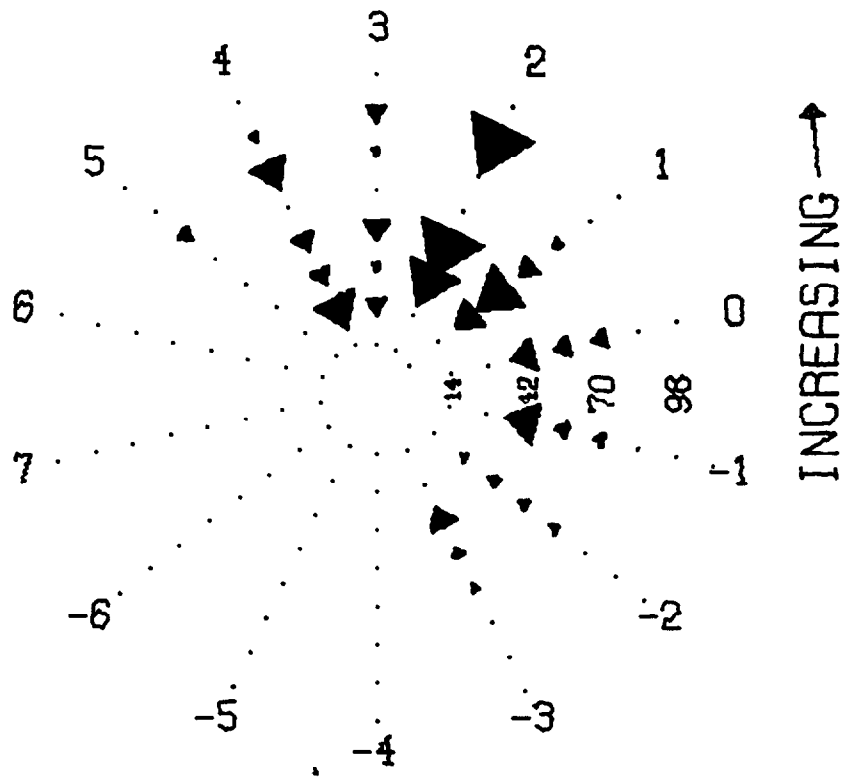


FIGURE 5B

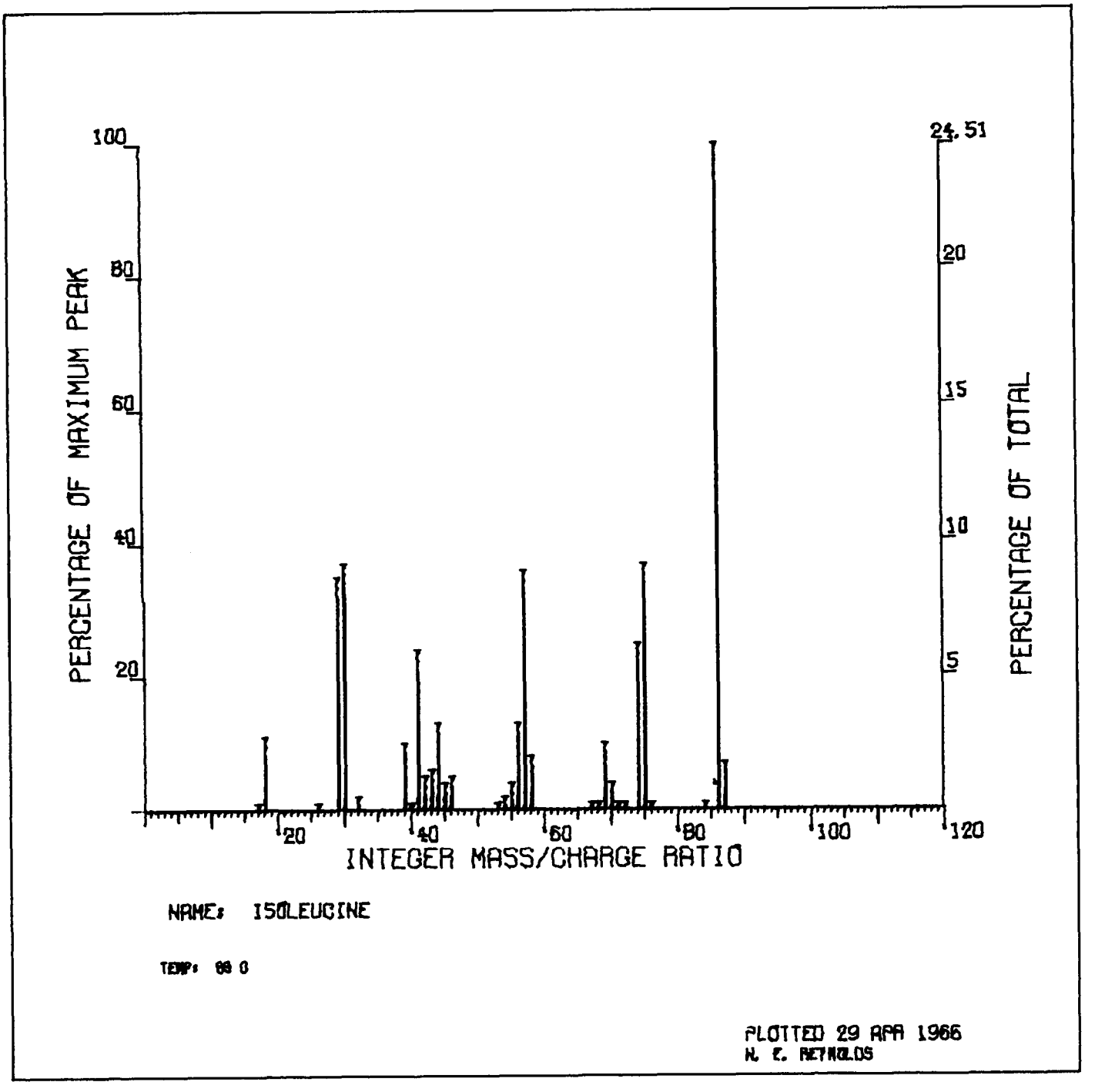
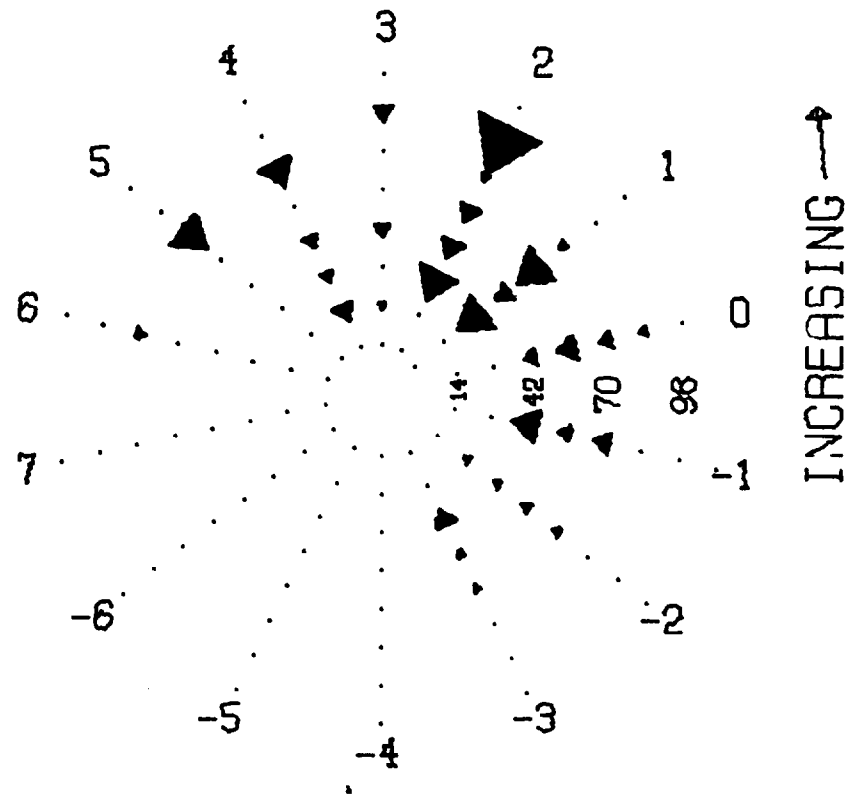

PERCENTAGE OF MAXIMUM PEAK
100
80
60
40
20
24.51
20
15
10
5
PERCENTAGE OF TOTAL
20
40
60
80
100
120
INTEGER MASS/CHARGE RATIO
NAME: ISOLEUCINE
TEMP: 60 C
PLOTTED 29 APR 1966
M. E. REYNOLDS

FIGURE 6A

NAME: ISOLEUCINE



TEMP: 83 C

FIGURE 6B

d. Computer Manipulation of Chemical Hypotheses.

The DENDRAL system has now been fully implemented, with respect to acyclic structures. The functions have been written in the LISP language, allowing the system to be run with only minor patching efforts on the IBM-7090, a PDP-6, and the Q-32 at System Development Corporation. The last is a time-sharing system with remote terminals--in the present case operated over a conventional teletype line some 400 miles long. With 46,000 words of available memory and a 1 microsecond cycle time, the Q-32 is only barely adequate even for the present complexity of the program and further finesse is strongly dependent on the gradually realized availability of more sophisticated computers. Programs of this complexity of artificial intelligence can use almost unlimited capacity, and certainly need a couple of magnitudes more than simple data-acquisition functions which can get by in a fashion with some few thousands of words of fast memory. Both applications will benefit from the development of improved techniques for automatic swapping of program and data blocks between different levels of memory, e.g., between magnetic core and drum, card, disc, or tape--a central issue in the currently developing time-sharing systems.

The present implementation is built around the DENDRAL canonical notation for organic structures. The functions will convert any arbitrary form into the canonical form. They will also generate an exact list of the isomers of a given structure. Finally, a number of rules of chemical plausibility have been inserted in order to confine the output to a set relevant to the immediate context. Finally a dialog for the insertion and development of this context information has been programmed. Nearing completion is a program to create a list, in order of plausibility, of the structures that might match a data set (a mass spectrum) i.e., to solve a typical problem of analytical organic chemistry. The main limitation here is the adequacy of the relevant theory, i.e., the rigorous deduction of the expected spectrum from a known molecule. The program is therefore being used in this theory-checking mode, i.e., to perfect the specifications that can lead to an expected spectrum closer to what has been observed. The tedious bookkeeping needed has been a serious deterrent to quantitating the rules of molecular fragmentation as observed in mass spectrometry.

The DENDRAL system has also been specified for cyclic structures, but a complete implementation is probably not feasible with the present generation of computers.

That is, it would take hours to compute all the isomers even of relatively simple molecules. However, some preparatory theoretical work has been completed, e.g., to perfect the notational system so that it is clearly precise and exhaustive. This has involved an excursion into the theory of cyclic graphs, and a proof that the smallest non-Hamiltonian polyhedron has at least 22 (probably 38) vertices (in cooperation with Professor V. Klee and Mr. D. Barnett of the University of Washington). This would be the smallest graph that would cause any embarrassment to the DENDRAL system (and a non-fatal one at that). However almost all practical contingencies can be met with a rather small set of cyclic graphs, and these are being grafted onto the DENDRAL system, together with some elementary manipulations for simulating chemical group reactions.

Part of the probem of memory size has some amusing human analogies. The solution of any problem can be broken up into a recursive set of sub-problems, many of which have been solved previously. To avoid spending a prohibitive amount of processor time in repeating work already done before, memory space is invested in saving automatically the results of all novel sub-problem solutions. But as the program thus becomes more sophisticated, it rapidly uses up free memory storage and, as the LISP system works, this eventually slows up the computation to the point of great sluggishness. The solution (like the human one, as culture accumulates) is to put away the routine savings in a library in higher level, slower access memory, and call in only that 'literature' currently needed for the immediate sub-problem. However, some finesse is still needed to optimize the number of books held out of the library, and to facilitate making and changing this decision from moment to moment.

A very serious problem arises when some of the axioms must be changed, as it proves to be very difficult to revise the traditional library, which contains many items many steps away from those axioms. No completely reliable method of averting the costly need to wipe out the library and start fresh has so far been found (usually too painful for humans to contemplate). However, the system was not originally designed with this contingency in mind, and is being reviewed. Needless to say a memory-dependent system is also exquisitely sensitive to any errors in computation, which will be rapidly propagated, often without being readily detected. These problem-solving systems have already passed the threshold of complexity which justifies leaving them run unattended without some built-in feedback to monitor the plausibility of the deductions. However,

machine errors remain very rare compared to startling lapses in the software found only when novel problems are encountered.

V. Computer Managed Instrumentation

The first steps in the hardware realization of computer managed instruments as applied mainly to gas chromatography and mass spectrometry have been taken. Some of this effort consists of simply data acquisition or better data acquisition systems, some of improved interface hardware such as log amplifiers and some as feedback control of experiments.

a. Gas Chromatography

Gas chromatographic data curves are now being read and integrated by the LINC computer. This is accomplished by a curve follower utilizing the California Computer Products plotter. Areas indicated by the investigator are computed and written out on a teletype with an appropriate notation being made on the strip chart by the Cal. Comp. plotter in its pen mode. About 800 charts have been processed to date using this system. Interface hardware has been developed to permit programming a temperature gradient control signal for use on the gas chromatographs. Appropriate buffer amplifiers and cabling have been installed to take the gas chromatograph output data directly into the computer. The data gathering capability of this system has been demonstrated but the software package has not yet been completed.

b. The LINC Computer

The uses of the LINC computer as a laboratory tool are still being pursued with considerable effort and interest. Data acquisition and feedback control of experiments are the main line of endeavor. The computer is routinely used to provide both functions for the nanosecond flash fluorometer.

The use of the LINC as an input device for a large time-sharing system (ACME) is being investigated.

A utility system has been developed to generate IBM tape in our laboratory. This is to provide continuous data recording of analog to digital converted data at sample rates of 445 or 4,450 samples per second. Up to approximately 2 1/2 million samples may be stored. It also permits conversion of any LINC format tape to IBM format tape. This system is currently employed in directly taking

data from the Bendix time-of-flight master chromometer. It is also employed by Dr. Stryer in his research activities.

c. Mass Spectrometer Data Acquisition

The log amplifiers previously developed have been modified to provide zero level following or null setting. This feature is now extended to all ranges of the log amplifier and has proved very desirable for use on the CH-4 mass spectrometer.

Some preliminary design and breadboard work has been done on improving the data acquisition for the Bendix time-of-flight. The Bendix time-of-flight system is now being modified so that direct control of its adjustments can be made by the computer.

A great deal of work has been done studying the computer acquisition of high resolution data from the MS-9 mass spectrometer. This work has been coordinated with the work of the manufacturer and the investigation being carried out at JPL by Dr. Norman Horowitz' group in addition to cooperation with the Stanford Chemistry Department.

VI. UV Microspectrophotometry

Our efforts on video scanning techniques, which have been described in previous reports, are continuing with emphasis in their application to the data readout of a Model E (Beckman) ultracentrifuge.

Initial efforts of data readout on the ultracentrifuge have been successful.

The pulsed light source system has been installed in the centrifuge and the appropriate synchronization hardware incorporated to give a versatile system. In its present condition a rotor with a maximum of four cells, each cell containing two samples, can be scanned sequentially. Preliminary results indicate that the signal to noise ratio is about ten to one and the spatial resolution as good as presently used film densitometry. Certain problems, however, have been encountered with modulating the short arc Xenon lamp. These problems were not evident in dc operation. They include arc instability, acoustical resonance, and mechanical variances. These cause the lamp to be extinguished at high repetition rates (maximum of 1^{1kc} and 100mjoules power).

Laboratory experimentation has shown that cooling the lamp allows the lamp to remain lit even at higher modulating frequencies required.

We are in the process of installing a cooling system in the light source associated with the ultracentrifuge. Further evaluation of the entire U.V. densitometry system is continuing.